

# HYDROGEN ION CONCENTRATION IN THE BLOOD OF CERTAIN INSECTS (ORTHOPTERA).

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Inasmuch as most accurate quantitative results on the hydrogen ion concentration of blood and body fluids have been largely confined to higher animals it seems highly desirable to extend these observations to lower forms, and especially insects. Since only small amounts of fluids are usually available from single insects, an accurate micro-electrometric method is necessary for the successful carrying out of such investigations. A simple micro hydrogen electrode and vessel, particularly designed for this purpose, have been described by the author (Bodine and Fink, 1). The present paper contains data obtained by the use of this method for the blood of different species of grasshoppers.

With the exception of two papers (Bishop, 2; Brecher, 3), all previous work on insect blood seems to have been carried out by means of colorimetric methods (Jameson and Atkins, 4; Crozier, 5; Bodine, 6; Glaser, 7). Since the blood of most insects is colored it seems highly desirable that other methods than micro-colorimetric ones be used and especially since the indicator most used in these methods is brom-thymol-blue. The blue-green to yellow-green color of insect blood seems to introduce an undesirable factor in micro-colorimetric methods using this indicator. Results obtained by the use of colorimetric methods by different authors are somewhat variable and it therefore seemed highly desirable to check them by means of an electrometric method.

In the present investigation different species of grasshoppers, reared under controlled food, temperature and other laboratory conditions, have been used as well as other animals taken from their normal out-of-door environments. Individual studies have been made on the blood of certain species from the day of hatching until death or in other words during their entire life cycle. Blood was taken from the grasshoppers in a variety of ways,—by

cutting off the tip of the feet, puncturing the animal by means of needles, insertion of electrode vessel directly into the body cavity, etc. No marked differences in pH of the blood obtained by these different methods was noted. The method found most satis-

TABLE I.

SPECIES: *Chortophaga viridifasciata*.

Age, Color, Sex.	pH of Blood.
nymph—brown—♂	6.83
nymph—green—♀	6.83
nymph—brown—♀	6.84
nymph—brown—♀	6.86
adult—♂	6.62
adult—♂	6.43
adult—♂	6.67
adult—♂	7.05
adult—♂	6.85
adult—♀	6.65
adult—♀	6.94
adult—♀	6.51
adult—♀	6.95
adult—♀	6.73
adult—♀	6.93
Average	6.79
Range	6.43-7.05

*Explanation of Table I.*

Data taken at random from many individuals showing pH of blood of *Chortophaga viridifasciata*. Animals all taken from out-of-doors. Some bloods tested at once. Other animals kept in laboratory and fed lettuce over different periods. Samples of blood tested regularly. Average given for all individuals tested.

factory and least harmful to the grasshopper, however, was a small needle puncture into the mid-lateral abdominal wall. Only a very small amount of blood is thus lost by the insect and the wound is quickly sealed. Sufficient blood (approximately 0.01 cc.), however, is obtained in this way for electrometric determinations of pH values. The grasshopper was usually punctured and the blood immediately drawn up into the electrode vessel with practically no exposure to air. As a matter of fact, identical results were obtained with blood taken from the interior of the animal by the insertion of the electrode vessel directly into the animal as by the method of puncturing and then immediately

TABLE 2.

SPECIES: <i>Melanoplus femur rubrum</i> .		pH of Blood.
Age, Sex.		
Nymph	.....	7.00
Nymph	.....	6.23
Nymph	.....	6.95
Nymph	.....	6.36
Nymph	.....	6.70
Nymph	.....	6.59
Nymph	.....	6.71
Nymph	.....	7.07
Nymph	.....	6.79
Nymph	.....	6.96
Nymph	.....	6.55
Nymph	.....	6.70
Nymph	.....	6.45
Nymph	.....	6.66
Adult—♂	.....	6.86
Adult—♂	.....	6.86
Adult—♂	.....	6.58
Adult—♂	.....	6.80
Adult—♀	.....	7.00
Adult—♀	.....	6.73
Average	.....	6.70
Range	.....	6.23-7.11

*Explanation of Table 2.*

Data selected showing range of pH for blood of different individuals. All animals laboratory raised and fed lettuce. Average and range of pH for all individuals tested.

TABLE 3.

SPECIES: <i>Melanoplus differentialis</i> .		pH of Blood.
Age.		
Nymph	.....	6.85
Nymph	.....	6.43
Nymph	.....	6.52
Nymph	.....	6.56
Nymph	.....	6.50
Nymph	.....	6.75
Nymph	.....	6.98
Nymph	.....	6.93
Average	.....	6.68
Range	.....	6.42-6.98

*Explanation of Table 3.*

Data selected from several hundred individuals showing range of pH values. Adult values are similar to those for nymphs. Animals reared in laboratory and fed lettuce. Average and range of pH for all individuals tested.

drawing the blood up into the vessel. Individual adult grasshoppers can be punctured at least two to three times daily without any apparent injury.

TABLE 4.

*Romalea microptera*.<sup>1</sup>

Date.	Stage.	Average pH of Blood.
Feb. 19, 1926.....	Nymphs	6.83
" 26, 1926.....	Nymphs	6.88
Mar. 5, 1926.....	Nymphs	6.72
" 12, 1926.....	Nymphs	6.74
" 19, 1926.....	Nymphs	6.84
" 26, 1926.....	Nymphs	6.79
Apr. 9, 1926.....	Nymphs	6.66
" 16, 1926.....	Nymphs	6.87
" 23, 1926.....	Nymphs	6.56
" 28, 1926.....	Nymphs	7.10 (?)
" 30, 1926.....	Adults	6.82
May 7, 1926.....	Adults	6.88
" 14, 1926.....	Adults	6.84
" 21, 1926.....	Adults	6.68
" 28, 1926.....	Adults	6.46
June 1, 1926.....	Adults	6.77
" 2, 1926.....	Adults	6.67
" 3, 1926.....	Adults	6.55
" 4, 1926.....	Adults	6.53
" 5, 1926.....	Adults	6.72
" 7, 1926.....	Adults	6.50
" 9, 1926.....	Adults	6.59
" 11, 1926.....	Adults	6.82
" 15, 1926.....	Adults	6.87
Average.....		6.73
Range.....		6.4-6.8

*Explanation of Table 4.*

Detailed average pH values for grasshoppers from day of hatching until death. Data for each day represents average readings for from 5 to 20 individuals. All animals laboratory raised and fed lettuce. Average and range of pH given for all individuals tested.

All laboratory animals were hatched from eggs laid in the laboratory and were fed lettuce. Animals were also collected

<sup>1</sup> The author is indebted to Dr. H. Fry for his generosity in supplying some of the eggs used in these experiments. These eggs were from a cross between *Romalea microptera* and *Romalea microptera*, variety *macri*. No differences in pH of the blood from this cross and the *R. microptera* were noted.

from the outside and comparisons made with those reared entirely under laboratory conditions.

Temperature during the course of the experiments ranged from 22 to 24° C.

TABLE 5.

Temp. 24° C.—June 15, 1926.

	Samples—E.M.F.	Average pH.
Buffer (5.65).....	(a) .57573	5.61
	(b) .57580	
	(c) .57560	
Romalea adult.....	(a) .65250	6.90
	(b) .65555	
Buffer (5.65).....	(a) .58210	5.69
	(b) .58088	
	(c) .58006	
Romalea adult.....	(a) .64553	6.79
	(b) .64553	
Buffer (5.65).....	(a) .58173	5.67
	(b) .57953	
Romalea adult.....	(a) .65203	6.90
	(b) .65273	
Buffer (5.65).....	(a) .58265	5.67
	(b) .57963	
	(c) .57963	
Romalea adult.....	(a) .65226	6.89
	(b) .65163	
Buffer (5.65).....	(a) .58200	5.67
	(b) .57742	

*Explanation of Table 5.*

Part of daily record for June 15, 1926, to show method of procedure in making determinations of blood pH values. Note that standard or buffer solution is always read before and after every reading on blood as check on electrode.

The results of the determinations are summarized in Tables 1-5. From an examination of these it is evident that considerable variation exists in the hydrogen ion concentration of the blood of the same and different species. No marked species, sex or age differences have been detected. The variations shown can hardly be due primarily to differences in food conditions since the laboratory animals were all fed the same food, lettuce, and the out-of-door animals had varied diets depending on the locality from which they were taken. The data in the tables are summarized and do not show individual variations which are to a degree

closely similar to those indicated for groups. Readings for the same individual, however, are quite constant for any one day. An explanation for these consistent variations is not at hand.

It is of some interest to compare the present results with those obtained with colorimetric methods by Glaser (7), for grasshoppers (*Melanoplus differentialis*). This author found the range of pH values for the blood of these grasshoppers to be 7.2 to 7.6. In previous experiments (6), using a colorimetric method the average pH for the blood of many species of grasshoppers was found to be 6.8. It seems to the author that the results of Glaser (7) are too high, due doubtless to difficulties in the colorimetric method already indicated above.

Preliminary experiments on the CO<sub>2</sub> tension of grasshopper blood indicate a rather low value, which seems consistent with an acid pH for the blood and the mechanism of tracheal respiration.

Grasshopper blood seems to be slightly buffered, since upon dilution no marked changes in pH are detected until a dilution of two to four times is reached. Most of the buffer effects are apparently due to the fairly large amounts of phosphates which the blood contains. In contrast to mammalian blood there seems to be present rather small amounts of carbonates.

Results for pH values of blood for other insects obtained by means of electrometric methods (Bishop, 2; Brecher 3) 6.8, agree rather closely with those obtained in the present work for grasshopper blood.

#### SUMMARY.

The pH values for the blood of different species of grasshoppers have been determined by a micro-electrometric method. Considerable variation exists in the pH values for blood of the same species of animals. No correlations seem to exist between blood pH and age or sex. The range and average pH values for the blood of the different species of grasshoppers examined are as follows:

Species.	Range of pH.	Average pH.
<i>Melanoplus femur rubrum</i> .....	6.4-6.8	6.73
<i>Melanoplus differentialis</i> .....	6.42-6.98	6.68
<i>Chortophaga viridifasciata</i> .....	6.43-7.05	6.79
<i>Romalea microptera</i> .....	6.4-6.8	6.73

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